Figure S1, related to Figure 1. Translational recovery during chronic ER stress is independent of eIF2B activity, changes of five eIF2B subunits levels, eIF5 protein, and translation elongation rate.

(A) Translational recovery in response to tunicamycin (Tm)-induced ER stress in MEFs is independent of eIF2B activity. Western blot analysis for the indicated proteins from total cell lysates of wild type and eIF2α$^{S51A/S51A}$ MEFs treated with tunicamycin (3 μM) for the indicated times. (B-C) MEFs were treated with tunicamycin (Tm) for the indicated times and either protein synthesis was measured by [^{35}S]Met/Cys incorporation into proteins (B), or eIF2B GEF activity measured in cell extracts (C). The mean ± S.E.M. of triplicate determinations is shown. * $p <$ 0.01; *n.s.,* not significant.

(D) Absence of recovery of eIF2B GEF activity during chronic ER stress is not caused by decreased levels of the five eIF2B subunits. Western blot analysis for the five subunits (α, β, γ, δ, ε) of eIF2B from total cell lysates of wild type MEFs treated with Tg (400 nM) for the indicated time.

(E) eIF5 is not required for efficient translation of the ATF4 mRNA and protein synthesis recovery during chronic ER stress. MEFs were infected with lentivirus expressing either control shRNA (shCon) or shRNA against eIF5 (sheIF5), followed by 3 days of puromycin selection. Western blot analysis for the indicated proteins, and protein synthesis measured by [^{35}S]Met/Cys incorporation into proteins in MEFs of shCon and sheIF5 treated with Tg (400 nM) for the indicated times. The mean ± S.E.M. of triplicate determinations is shown. * $p < 0.01$

(F) Translational recovery in chronic ER stress is independent of elongation rates. MEFs were treated with Tg (400 nM) for 1 h and 12 h, followed by the measurement of ribosome half-transit times. *PMS*, post-mitochondria supernatant; *PRS*, post-ribosome supernatant.

Figure S2, related to Figure 1. Polysome profiles of mRNAs during ER stress.

MEFs were treated with Tg (400 nM) and PERKi (2 μM) for the indicated times, and specific mRNA distributions on fractionated sucrose gradients (15%-50%) was determined. RNA was extracted from individual fractions and a cDNA pool was generated. Specific mRNAs were analyzed by qPCR. The *dashed lines* indicate light (fractions 7-9) and heavy (fractions 10-12) polysomes. Percentage of specific mRNA distributions in the light and heavy fractions are shown. The association of specific mRNAs with light or heavy polysomes was determined as a percentage of the polysome-associated mRNA signal over the total mRNA signal in each polysome profile.

Figure S3, related to Figure 3 and Figure 4. Recovery of protein synthesis during chronic ER stress is eIF4F-independent but eIF3-dependent.

(A) Recovery of protein synthesis during chronic ER stress is less dependent on eIF4A activity. Protein synthesis measured by [^{35}S]Met/Cys incorporation into proteins in MEFs (WT or eIF2$\alpha^{S51A/S51A}$) treated with Tg and the eIF4A inhibitor hippuristanol (250 nM) or its vehicle (DMSO) as indicated. Hippuristanol was added for 1 h after completion of the indicated Tg treatments. Data were normalized to their own controls.

(B) eIF4E2 is not required for protein synthesis recovery during chronic ER stress. Wild type (eIF4E2^{WT}) and eIF4E2 deficient (eIF4E2^{KO}) MEFs were treated with Tg (400 nM) for the indicated times, followed by protein synthesis measurement of [^{35}S]Met/Cys incorporation into proteins. The mean ± S.E.M. of triplicate determinations is shown. * $p < 0.01$; *n.s.,* not significant.

(C-D) eIF3l is not required for efficient translation of uORF mRNAs and protein synthesis recovery during chronic ER stress. MEFs were infected with lentivirus expressing either control shRNA (shCon) or shRNA against eIF3l (sheIF3l), followed by 3 days of puromycin selection. (C) RT-qPCR evaluation of eIF3l knockdown efficiency and protein synthesis measured by [^{35}S]Met/Cys incorporation into proteins, and (D) Western blot analysis for the indicated proteins in MEFs of shCon and sheIF3l with Tg (400 nM) treatment for the indicated times. The mean ± S.E.M. of triplicate determinations is shown. * $p < 0.01$**Word did not find any entries for your table of contents.**

(E) Depletion of eIF3d inhibited translational reprogramming during chronic ER stress. MEFs were infected with lentivirus expressing shRNA against eIF3d (sheIF3d), followed by 3 days of puromycin selection. Polysome profiling was analyzed for untreated (control) and Tg-treated MEFs for 16 h. The association of specific mRNAs (ATF4, GADD34, and BiP) with light and heavy polysomes was analyzed using RT-qPCR as described in Figure S2.

Figure S4, related to Figure 4. Factors not involved in protein synthesis recovery during chronic ER stress.

MEFs were infected with lentivirus expressing either control shRNA (shControl) or shRNA against the indicated target genes, followed by 3 days of puromycin selection. Specific mRNA target knockdown efficiency was measured by Western blot or RT-qPCR analysis. In the knockdown of eIF2A, eIF4G2(DAP5), PDCD4, METTL3 and FTO, additional proteins were also measured by Western blotting, as indicated. Protein synthesis was measured by [^{35}S]Met/Cys incorporation into proteins in the indicated cell types treated with Tg (400 nM) for the indicated times. The basal level of protein synthesis rate in each knockdown was set as 100%, respectively.

Figure S5, related to Figure 6. Supplemental information for the genome-wide polysome profiling.

(A) PCA analysis of r-log normalized gene expression data of 48 samples used for genome-wide polysome profiling. Samples are colored according to treatment and symbols indicate cytosolic or polysome-associated origin of mRNA. (B) Translational efficiency (as calculated by anota)(Larsson et al., 2011) of selected genes compared to the control condition. (C) Coding sequence (CDS) length *vs.* residuals from a linear regression of polysome-associated mRNA on cytosolic mRNA at Tg:0h. Slope and *P*-value are indicated. (D) Residuals from a regression of cytosolic mRNA on mRNA length for mRNAs differentially translated during the acute phase (top row) and for genes that change congruently during the chronic phase (bottom row). D: down-regulated; N: non-regulated; U: up-regulated. (E) Regression of polysome-associated mRNA levels on cytosolic mRNA levels during Tg:0h (left) or Tg:1h (right). mRNAs significantly regulated during the acute or the chronic phase are indicated. (F) Scatterplots of log2 fold changes comparing Tg:16h+PERKi to Tg:16h using data from cytosolic or polysome-associated RNA. Genes with differential translational efficiency at Tg:1h *vs.* Tg:0h (left) and congruently changes at Tg:16h *vs.* Tg:1h (right) are indicated.

Figure S6, related to Figure 7. PERK inhibition during chronic ER stress promotes "foamy cell" phenotype

(A) PERKi-dependent mRNA translation during chronic ER stress promotes the "foamy cell" phenotype, a hallmark of ER dysfunction. Phase contrast images of MEFs treated with Tg (400 nM), Tm (3 μM), PERKi (2 μM), and protein synthesis inhibitors (cycloheximide at 25 μg/mL; hippuristanol at 1 μM; harringtonine at 2 μg/mL) for the indicated times. For PERKi treatment and co-treatment of protein synthesis inhibitors with PERKi, cells were treated with Tg or Tm alone for 12 h, and then inhibitors were added for an additional 12 h in the presence of Tg or Tm. Representative images are shown in 40 × magnifications.

(B) PERK inhibition during chronic ER stress leads to accumulation of ubiquitinated proteins. Western blot analysis for the indicated proteins from extracts of MEFs treated with Tg (400 nM) and PERKi (2 μM) for the indicated times.

(C) PERK activity inhibits the development of foamy cells during chronic ER stress in a manner dependent on the reprogrammed ISR. Phase contrast images of $eIF2\alpha^{S51A/S51A}$ and $PERK^{-/-}$ MEFs treated with Tg (400 nM) and PERKi (2 μM) for the indicated times. Experimental scheme is as shown in (Figure S6A). Representative images are shown in 40 × magnifications.

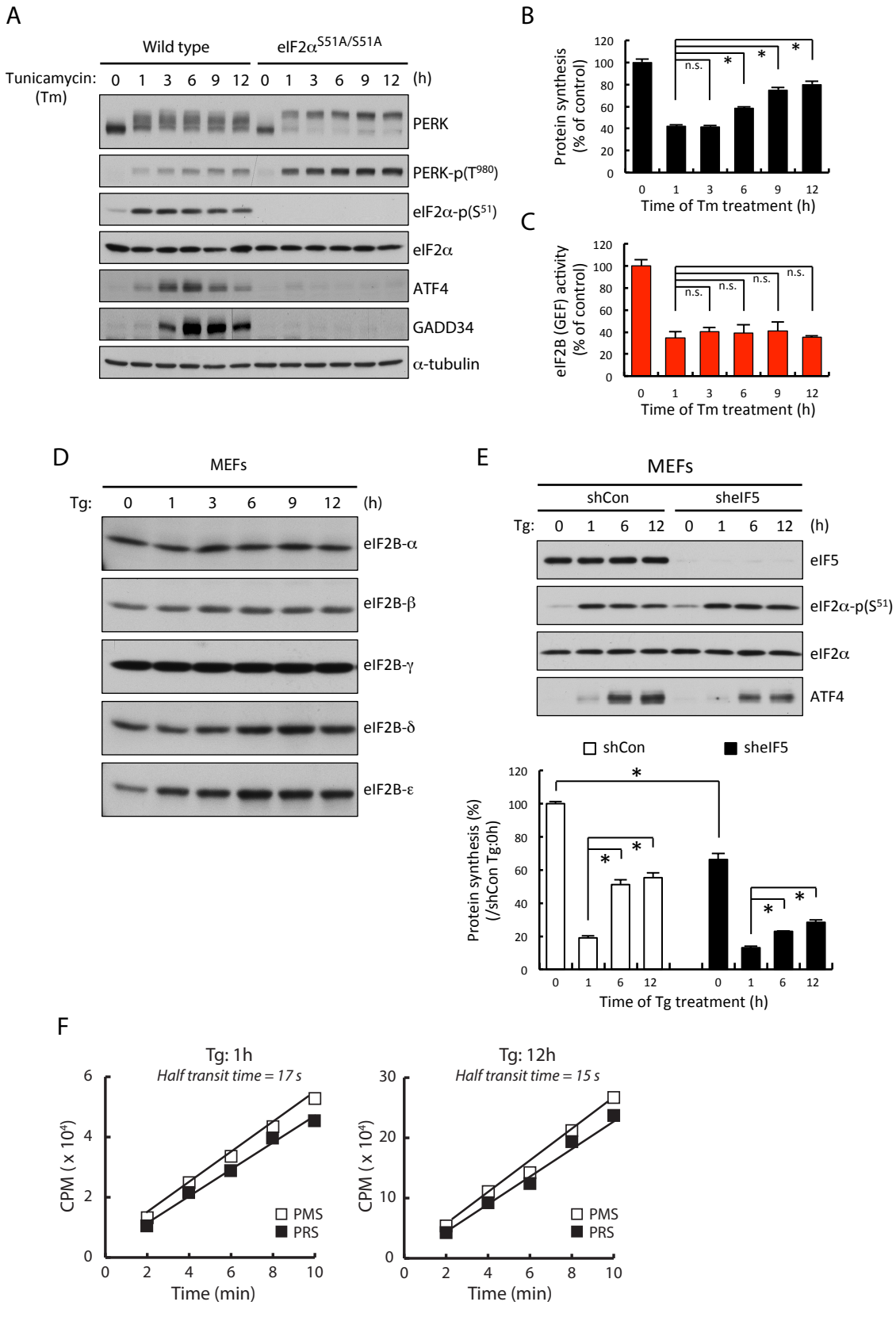

Figure S1, related to Figure 1.

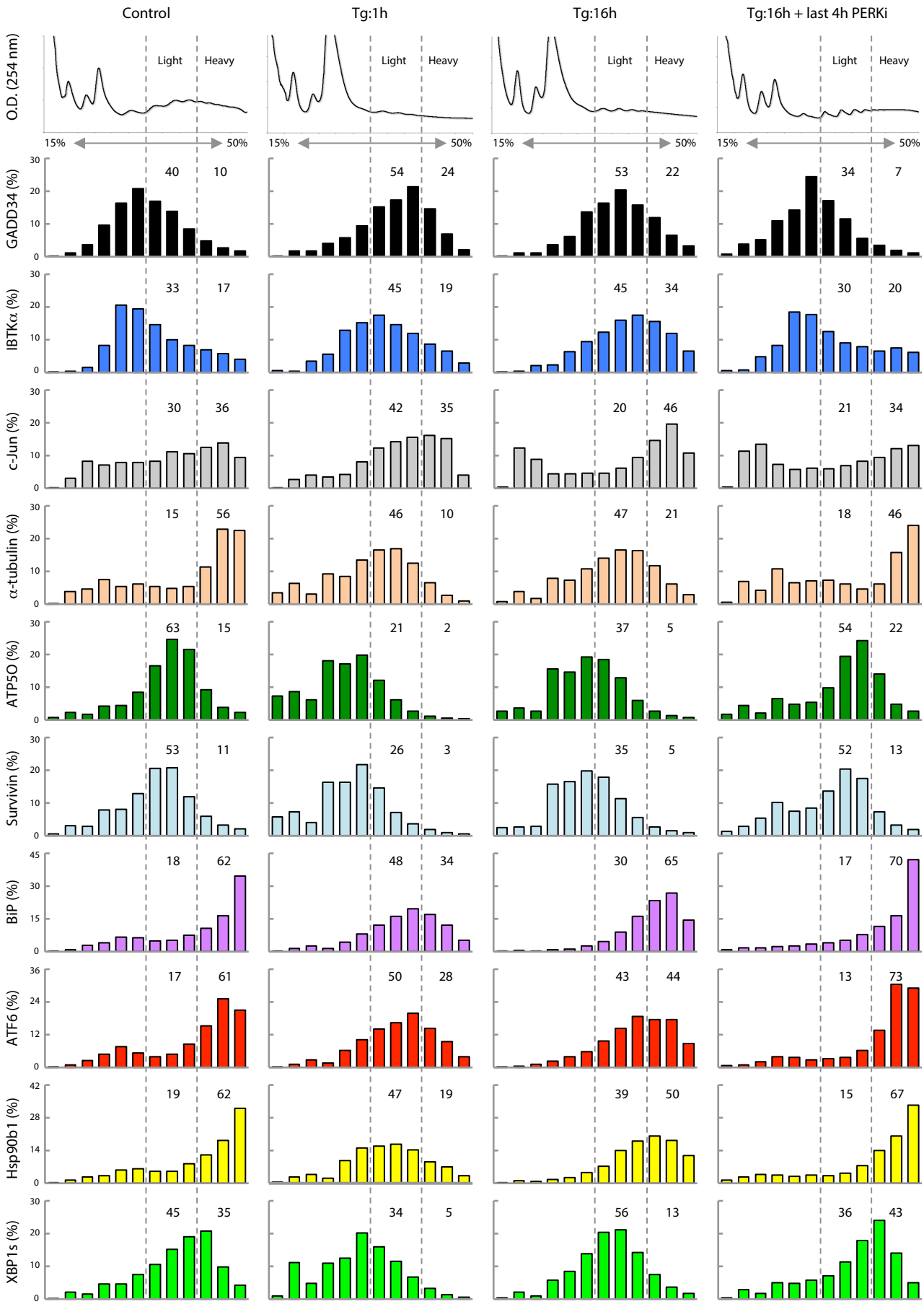

Figure S2, related to Figure 1.

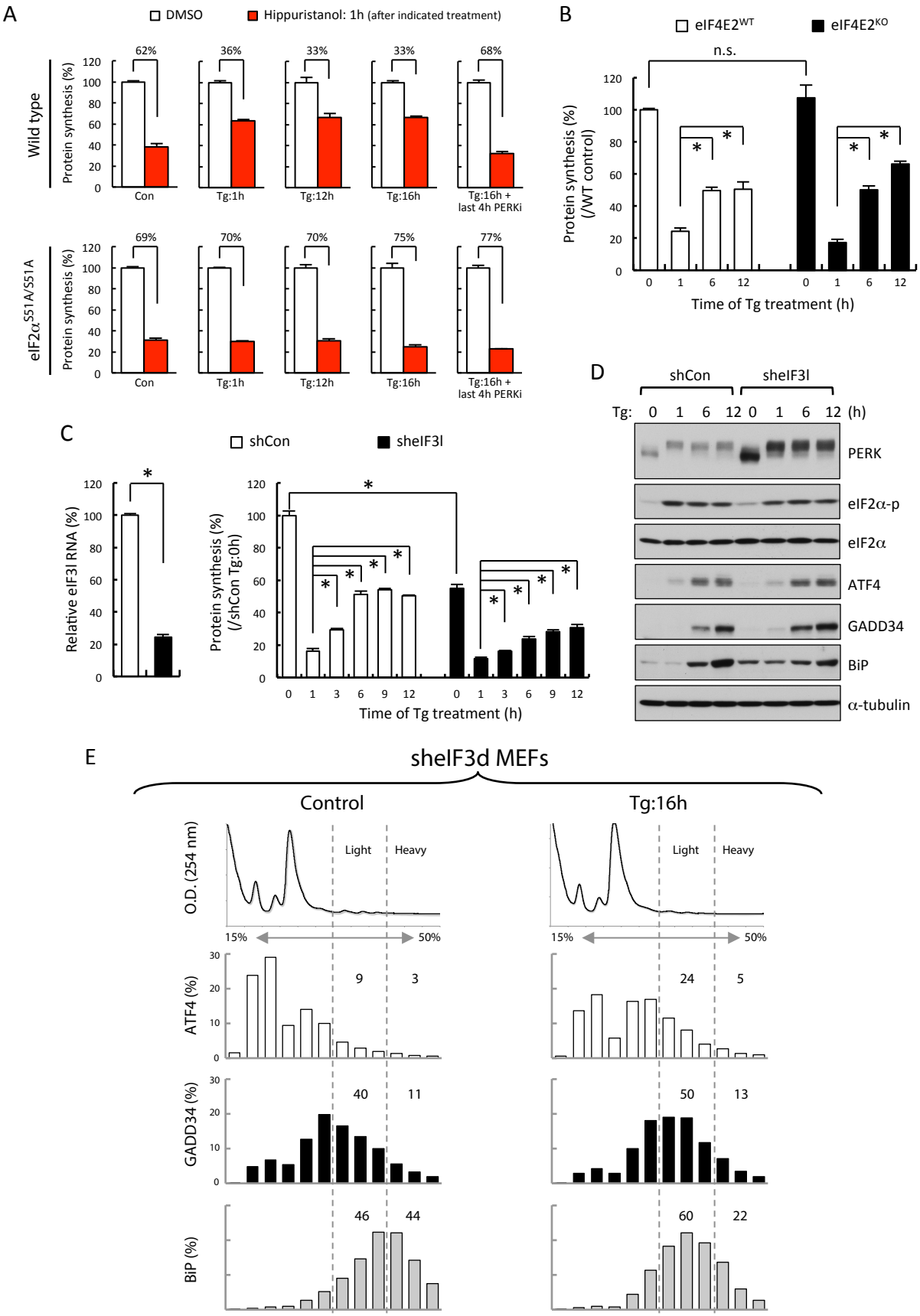

Figure S3, related to Figure 3 and Figure 4.

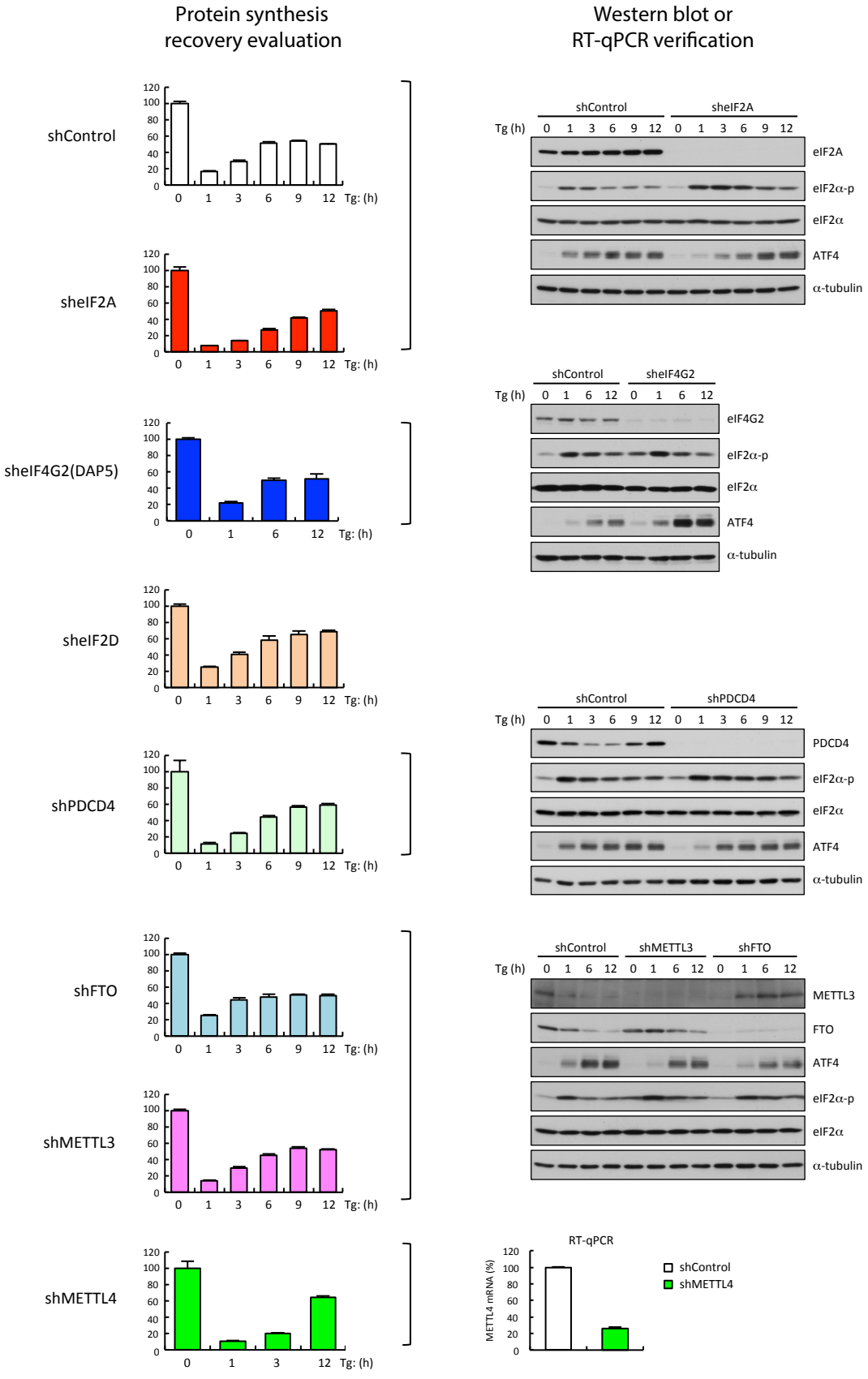

Figure S4, related to Figure 4.

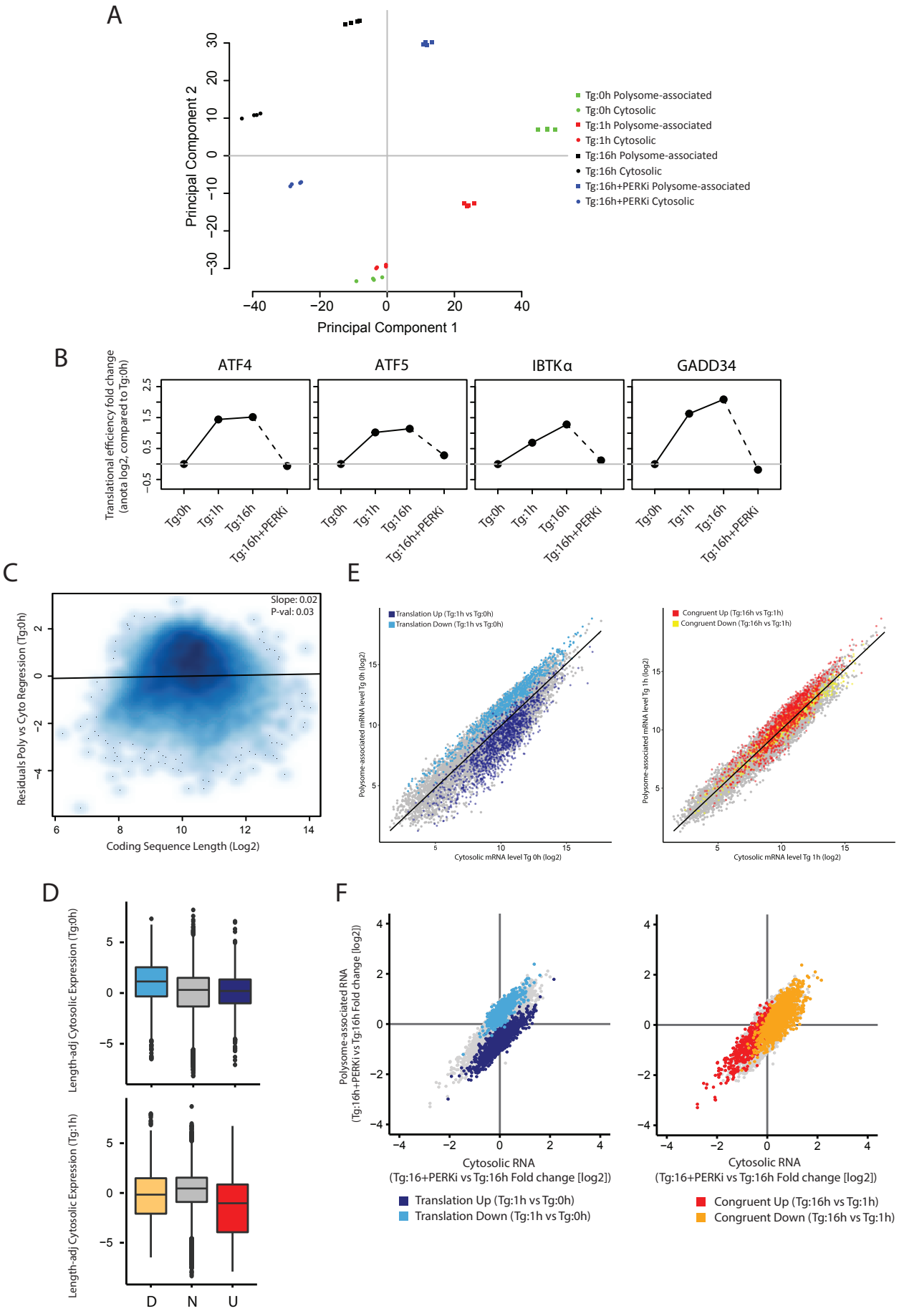

Figure S5, related to Figure 6.

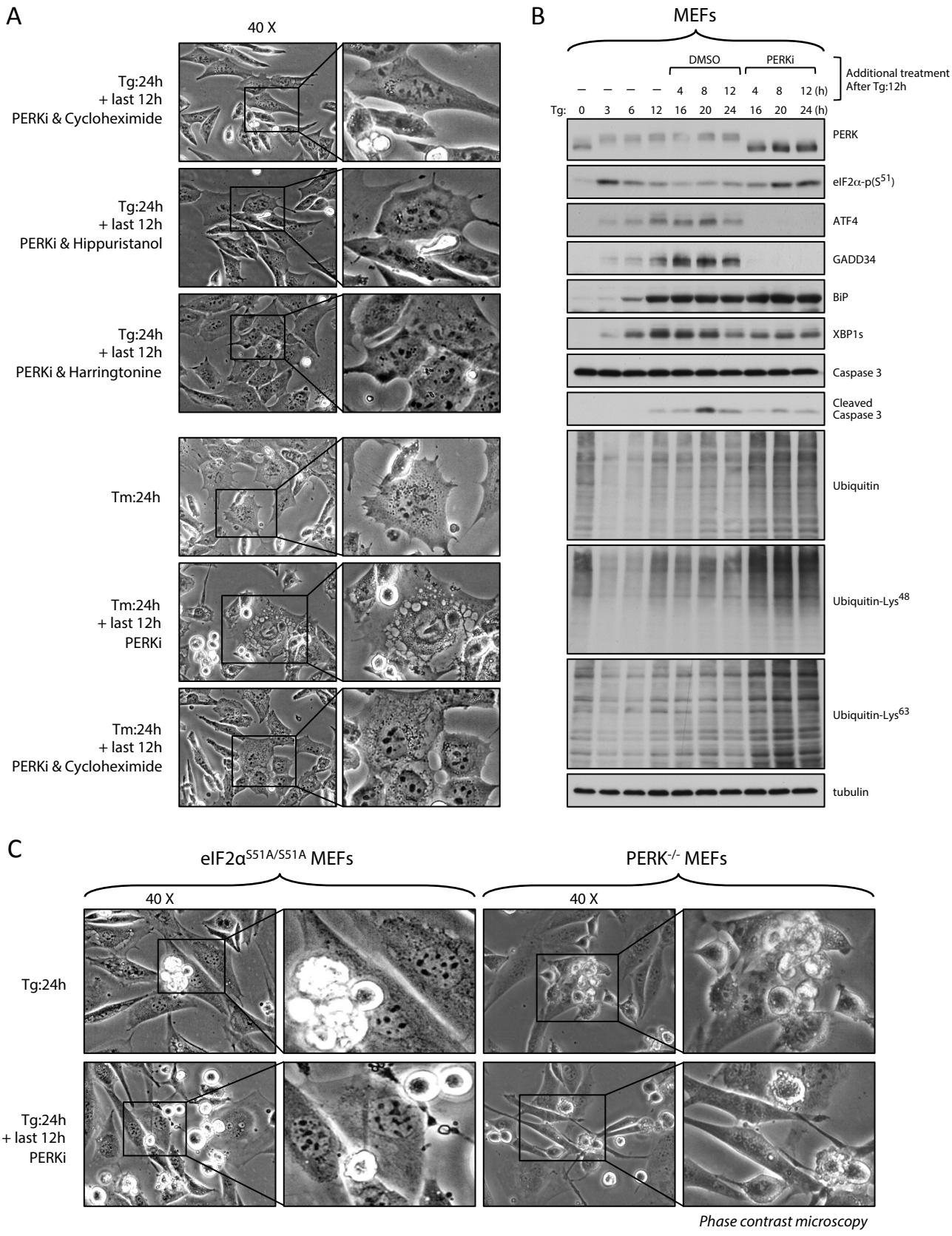

Figure S6, related to Figure 7.

Table S1, related to Figure 7. ER protein processing pathway (35 genes) identified from Tg:16h *vs.* Tg:1h genome-wide analysis

Protein processing in ER		Transcription factor target*				Cytosolic effect**		Polysome-associated effect***		Translation effect****	
Symbol	EntrezID	ATF4	CHOP	ATF6	XBP1s	(Log2)	FDR*****	(Log2)	FDR	(Log2)	FDR
Ddit3	13198					1.504833377	3.9567E-13	1.424008883	2.93755E-14	1.293683225	0.004770538
Derl1	67819					0.818216556	0	0.840434012	0	0.746108253	0.053692123
Derl2	116891					0.841392414	1.03888E-11	0.763585392	1.50659E-11	0.768010583	0.013949945
Dnajb11	67838					0.749247063	3.41713E-11	1.012137335	5.26485E-13	1.173436801	0.000652165
Dnajc3	100037258					1.420414096	3.07088E-13	1.629948094	6.697E-15	1.78729421	0.00097198
Edem1	192193					0.875890427	5.04932E-11	0.65995366	1.78313E-10	0.731551937	0.011007099
Edem2	108687					0.680234282	2.53596E-11	0.646173379	2.2369E-12	0.717505272	0.001216153
Edem3	66967					0.636239053	9.33706E-08	0.590798497	1.22453E-08	0.601359	0.004883363
Ero1l	50527					1.001329709	6.84055E-11	1.000076384	1.49511E-11	0.968397641	0.007081321
Ero1lb	67475					1.529500622	6.18677E-13	1.617822768	1.12884E-14	1.865424388	0.000673836
Park2	50873					1.266855542	1.01173E-07	1.200325192	4.2651E-06	1.169999872	0.064505671
Sec23b	27054					0.91051567	1.52216E-12	1.340044694	0	1.276546759	9.48966E-05
Stt3a	16430					0.616523029	1.46112E-08	0.790327886	1.42426E-10	0.83922761	0.001024782
Stt3b	68292					0.610726421	2.79216E-09	0.713906207	1.28207E-10	0.677476773	0.004255457
Sec61a1	53421					0.644131372	1.43641E-09	0.998879355	2.93755E-14	1.095003366	2.72606E-05
Vimp	109815					1.269165075	0	1.165429099	0	1.684898343	6.60479E-05
Wfs1	22393					1.151226168	2.2909E-09	1.389816663	2.31349E-10	1.64280821	0.001576616
Atf6	226641					1.118981394	1.25501E-12	1.198148368	9.54478E-14	1.465227019	0.000814936
Canx	12330					0.665779535	1.47992E-08	0.917002184	1.82838E-11	0.987565046	0.00029709
Calr	12317					1.241804754	1.65708E-14	1.705468663	0	1.902853081	0.000755503
Ern1	78943					0.679724728	2.15587E-10	0.856671325	3.6343E-13	0.871777783	0.000332303
Hspa5	14828					1.480120093	2.37887E-14	1.979469517	0	2.091711875	0.000561507
Hsp90b1	22027					1.673247556	1.2394E-13	2.045171391	0	2.145944802	0.000729166
Herpud1	64209					1.350818418	0	1.756136602	0	1.590143222	0.001212445
Hyou1	12282					1.408272206	0	1.640525936	0	1.672156327	0.007367006
Pdia3	14827					0.967765789	2.3067E-11	1.523152933	1.65003E-14	1.666436791	0.000169922
Pdia4	12304					1.181498124	5.574E-13	1.663374423	0	1.782960147	0.000832259
Pdia6	71853					1.200317514	0	1.600526206	0	1.412153522	0.001318473
Rnf185	193670					0.632691407	5.71755E-10	0.647983316	5.22241E-11	0.682617172	0.002759003
Sel1l	20338					0.940178078	6.04005E-12	1.144514571	0	1.133184272	0.000183263
Ssr1	107513					0.665128604	5.77443E-10	0.710814129	5.12972E-11	0.719308758	0.003740886
Ssr3	67437					0.889726908	1.57234E-10	0.629794584	1.48138E-09	0.582886689	0.039317364
Syvn1	74126					0.738149709	5.55377E-08	1.03908279	1.74331E-10	1.255779843	0.00020032
Tram1	72265					0.674198677	9.82196E-09	0.761243719	1.70588E-10	0.710555844	0.002758268
Ube2e1	22194					0.697423898	1.74585E-09	0.672615846	1.19663E-10	0.806072971	0.000927722

* Transcriptional target of ATF4 and CHOP is based on the CHIP database from Han et al., 2013.
Transcriptional target of ATF6 and XBP1s is based on the database from Shoulders et al., 2013.
** The changes in Cytosolic mRNA level from RNAseq readout.
*** The changes in polysome association level from RNAseq readout.
**** The overall translation status of each mRNA, combining cytosolic and polysome effect, calculated based on anota (Larsson et al., 2011).
***** FDR, *false discovery rate*, a multiple comparison adjusted *p*-value.

Table S2, related to STAR Methods.
Primers used for qPCR

Detected mRNA	Primer sense	Sequence
β-actin	For (-)	CTGGCACCACACCTTCTACAATG
	Rev (+)	GGTCATCTTTTCACGGTTGGC
ATF4	For (-)	GTTTGACTTCGATGCTCTGTTTC
	Rev (+)	GGGCTCCTTATTAGTCTCTTGG
ATF6	For (-)	CAGAGGCTCAAAGTCCCAAG
	Rev (+)	GAGATGCCTCCTCTGATTGG
ATP5O	For (-)	AAGCTTGTAAGGCCCCCTGT
	Rev (+)	GTGCGCTTGATGTAGGGATT
BiP	For (-)	ACTTGGGGACCACCTATTCCT
	Rev (+)	ATCGCCAATCAGACGCTCC
c-Jun	For (-)	CTGCAAAGATGGAAACGACC
	Rev (+)	CAGCTTGAGCAGCCCGACGTC
eIF3l	For (-)	CTTTGCCAACATCCTCCTGT
	Rev (+)	CAGCATCTTGTCCCCGTATT
IBTKα	For (-)	CCACCGTCTGCAGGATTATT
	Rev (+)	CTCGACCTTATCCGAATGGA
GADD34	For (-)	TACCCCTGTCTCTGGTAACCT
	Rev (+)	TGGCTTTGCATTGTACTCATCA
GAPDH	For (-)	CGCCTGGAGAAACCTGCCAAGTATG
	Rev (+)	GGTGGAAGAGTGGGAGTTGCTGTTG
Hsp90b1	For (-)	AGGGCGGAATCTTCTCCATTT
	Rev (+)	TTCTCTGTTGCTTCCCGACT
METTL4	For (-)	TCACCACAGCAGATAAAGCG
	Rev (+)	CCAACGGGAACACAAACTCT
Survivin	For (-)	ACCTTCAAGAACTGGCCCTT
	Rev (+)	CAGGGGAGTGCTTTCTATGC
α-tubulin	For (-)	CACTTACCACGGAGATAGCGA
	Rev (+)	ACCTTCTGTGTAGTGCCCCTT
XBP1s	For (-)	GAGTCCGCAGCAGGTG
	Rev (+)	CTGGGAGTTCCTCCAGACTA